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The Service Leader in Bioscience Testing

SPONSOR 14680

MRIaudio
2720 Loker Ave W
Suite N
Carlsbad, CA 92010
United States

217J0417H-M01G

STUDY NUMBER

17J0417H-M01G

REPORT DATE

November 27, 2017

FINAL REPORT

STUDY TITLE

Cytotoxicity – MEM Elution Test
(ANSI/AAMI/ISO 10993-5:2009)

TEST ARTICLE

MRIaudio Ear-Tips
Lot Number: 300-1
Part Number: 350

STUDY DIRECTOR

Jessica Renfandt
Senior Laboratory Technician
In Vitro Services

PERFORMING LABORATORY

Pacific BioLabs
551 Linus Pauling Drive
Hercules, CA 94547
United States

TABLE OF CONTENTS

SIGNATURE PAGE

This report is being submitted by the following personnel:

Study Director: Jessica Renfandt, Senior Laboratory Technician

11/27/17

X 

I approve the content of this document.
Signed by: Jessica Renfandt

RESPONSIBLE PERSONNEL

1. Adele Veridiano, Director, Microbiology
2. F. Michael Yakes, Ph.D., Executive Vice President
3. Tom Spalding, President

:mjc

STATEMENT OF COMPLIANCE

All aspects of the study contained in this report were conducted according to Pacific BioLabs Standard Operating Procedures and in compliance with the United States Food and Drug Administration (FDA) Good Laboratory Practice (GLP) for Nonclinical Laboratory Studies, Title 21 of the U.S. Code of Federal Regulations, Part 58 with the following exception(s):

The facility management was not able to assure the test article was appropriately tested for stability.

Study Director Signature

11/27/17

X 

I approve the content of this document.
Signed by: Jessica Renfandt

QUALITY STATEMENT

QUALITY ASSURANCE UNIT GLP MONITORING AND INSPECTION SUMMARY

In accordance with 21 CFR 58, this study, 17J0417H-M01G, was inspected by Quality Assurance at intervals adequate to assure the integrity of the study. The phase(s) of the study inspected, the date(s) of the inspection, QA auditor, and the date(s) that the QAU inspection report for this study were reported to the Study Director and Management are provided below.

Phase of Study	Date of Inspection	QA Auditor	Date QA Report Provided to Study Director and Management	Date QA Report Acknowledged by Study Director	Date QA Report Acknowledged by Management
Extraction	11/07/17	LM	11/08/17	11/08/17	11/09/17

The QAU inspection summary is routinely reviewed by the study director and management of Pacific BioLabs. Management is notified immediately if there are any deviations which might affect the integrity of the study data.

DATA/REPORT REVIEW

Quality Assurance has conducted a thorough review of the test data generated during this study. Report Number 17J0417H-M01G represents an accurate description of the conduct and final results of the study. To the best of my knowledge and ability, this study has been conducted in compliance with applicable Good Laboratory Practice regulations.

Date of Data/ Report Review	Date Review Provided to Study Director and Management	Date Review Acknowledged by Study Director*	Date Review Acknowledged by Management*
11/27/17	11/27/17	N/A	N/A

11/27/17

X

QA Review
Signed by: Sarah Lauder

*N/A = no findings reported to the Study Director or Management

STUDY SUMMARY

Purpose: The purpose of this test was to evaluate the cytotoxic potential of extracts of polymeric materials or any other materials intended to be implanted in the human body or that may come into contact with bodily fluids or injectable solutions. This test was performed according to Pacific BioLabs SOP 15B-01, which follow procedures outlined in ISO 10993-5 guidelines.

Procedures: The test article was prepared per Pacific BioLabs SOPs. Cell culture medium with serum solution (MEM) was used as the extractant. MEM without the test article was used as a reagent control. USP High Density Polyethylene Reference Standard was used as a negative control and Tygon F-4040-A was used as a positive control. The test article and controls were extracted with agitation at 37 ± 1°C for 24 ± 2 hours.

Multiple cultures of L-929 mouse fibroblast cells were prepared according to Pacific BioLabs SOPs. A minimum of three 10 cm^2 wells, each containing a monolayer of L-929 cells, was used per test article and control. After the 24 ± 2 hours extraction period, the growth medium from each of the triplicate 10 cm^2 wells was decanted and replaced with 2 mL of 1X Dulbecco's Phosphate Buffered Saline (PBS) to rinse away any debris. The PBS was then decanted and replaced with 2 mL of test article extract in each well. The control extracts were administered in the same manner as the test article extract. All cell culture wells were then incubated for 48 ± 2 hours at 37 ± 1°C in a humidified incubator with 5 ± 1% CO_2. The treated cells in all of the culture wells were examined under an inverted light microscope with 100X magnification after a minimum of 24 hours and 48 ± 2 hours following dose administration. The morphology of cells was observed at the 24 and 48 hour examination points according to the criteria stated in the ISO 10993-5 guidelines. The average score for the three test wells and controls at the 48-hour point was used to determine the cytotoxic response.

Interpretation: Based on ISO 10993-5 guidelines, cell morphology graded greater than 2 is considered to have a cytotoxic effect.

Results: Cells treated with the test article extract exhibited a response of grade 1 (slight reactivity) at 24 hours and a response of grade 2 (mild reactivity) at 48 hours.

Conclusion: Based on qualitative evaluation of the cells exposed to the test article extract, the test article was not considered to have a cytotoxic effect.

1. GENERAL INFORMATION

1.1. Study Dates

Study Authorization:	Signed Protocol
Dates Test Article Received:	October 20, 2017 and October 25, 2017
Study Initiation Date:	November 06, 2017
Date On Test:	November 08, 2017
Date Off Test:	November 10, 2017
Report Date:	November 27, 2017

1.2. Protocol

This test was conducted according to Protocol Number: 17J0417H-M01G (see Appendix II), which incorporates by reference Standard Operating Procedure 15B-01 and is on file at Pacific BioLabs. There were no amendments to the Protocol.

1.3. Deviations from Protocol

There were no deviations from the Protocol.

1.4. Key Personnel and Laboratories

Study Director:	Jessica Renfandt Senior Laboratory Technician Pacific BioLabs 551 Linus Pauling Drive Hercules, CA 94547 United States Phone: (510) 964-9000
Study Sponsor:	Joseph Caruso MRIaudio 2720 Loker Ave W. Suite N Carlsbad, CA 92101 United States Phone: (858) 266-8350

2. INTRODUCTION

Purpose: This procedure determines the *in vitro* biological reactivity of mammalian cell cultures to specific extracts or portions of samples prepared from the test material. The test was performed according to Pacific BioLabs SOP 15B-01, which follows procedures outlined in ISO 10993-5.

Justification of Test System: Mouse fibroblast cells were selected based on recommendations provided by ISO 10993-5 guidelines.

Justification for Selection of Media: Single Strength Minimum Essential Medium was selected based on recommendations provided by ISO 10993-5.

Justification of Route of Administration: The route of administration was based on ISO 10993-5 requirements for this test. The test article was extracted and administered to mouse L-929 cells via medium compatible with the test system.

Dose Rationale: The dose was selected based on recommendations provided by ISO 10993-5 guidelines.

3. MATERIALS AND METHODS

3.1. Test Materials

3.1.1. Test Article Identification

Test Article Name:	MRIaudio Ear-Tips
Physical Description:	Solid
Total Quantity Received for Testing:	Two jars, each containing ~50 pairs
Quantity Used for This Study:	Six pieces (received October 25, 2017)
Lot Number:	300-1
Part Number:	350
Sample Code:	Not provided by Sponsor
Expiration Date:	10/01/2018
Special Handling and/or Precautions:	None
Sterilization Data:	Non-Sterile
Storage Conditions:	Room Temperature
Final Intended Use/Application:	Used as an earbud for sound protection

3.1.2. Negative Control Article Identification

Name:	USP High-Density Polyethylene (HDPE) Reference Standard
Manufacturer:	USP
Physical Description:	Plastic
Quantity/Container:	34.0 cm^2 per pouch
Total Quantity Used for This Study:	One pouch
Lot Number:	K0M357
Expiration Date:	04/20/18 (assigned by Pacific BioLabs)
Sterility Status:	Sterile by Autoclave
Storage Condition:	Room Temperature

3.1.3. Positive Control Article Identification

Name:	Tygon F-4040-A
Manufacturer:	Saint-Gobain Performance Plastics
Physical Description:	Solid; Yellow plastic tubing
Quantity/Container:	37.4 cm^2 per pouch
Total Quantity Used for This Study:	One pouch
Lot Number:	21926463
Expiration Date:	02/02/18
Sterility Status:	Non-Sterile
Storage Condition:	Room Temperature

3.1.4. Test Article Characterization

Test Article: The Sponsor is responsible for all test article characterization specified in the Good Laboratory Practices (GLP) regulations (21 CFR 58.105). Because this is a solid material(s) containing no drug(s), characterization of the test article strength and purity are not considered applicable requirements. The Sponsor has not supplied sufficient information to Pacific BioLabs to assure characterization of the test article meets applicable requirements. Specifically, information that would allow for the evaluation of the stability of the test article (e.g., shelf life) was not provided. The absence of this information is considered a GLP violation and is noted in the compliance statement for this report. The Sponsor is responsible for maintaining records of manufacture that would provide information on the composition of the test article and would be able to supply those records if requested by regulatory authorities.

Negative Control Article: The negative control article was supplied by Pacific BioLabs and information related to the characterization of the control article can be found in Appendix I. The negative control article was adequately characterized as specified in the Good Laboratory Practices (GLP) regulations (21 CFR 58.105).

Positive Control Article: The positive control article was supplied by Pacific BioLabs and information related to the characterization of the control article can be found in Appendix I. The positive control article was adequately characterized as specified in the Good Laboratory Practices (GLP) regulations (21 CFR 58.105).

3.1.5. Test and Control Article Dose Solution Characterization

Test Article Dose: The test article was extracted by Pacific BioLabs according to ISO 10993-12. The resulting extracts were administered within 24 hours to the test system as specified by ISO 10993-12. Characterization of the extract for strength (concentration), homogeneity, or stability was not conducted. Post-extraction, the pH of the test article extract was measured at Pacific BioLabs and determined to be approximately 8.0. Compliance with the ISO 10993-12 stipulation for use of extracts within 24 hours of preparation is considered adequate to justify absence of additional information.

Negative Control Article Dose: The control article was used without modification. No further characterization of the negative control article, beyond that provided by the supplier, was conducted.

Positive Control Article Dose: The control article was used without modification. No further characterization of the positive control article, beyond that provided by the supplier, was conducted.

3.2. Test System

Name:	Fibroblast cell
Organism:	*Mus musculus*
Cell Line:	L-929 (ATCC cell line CCL 1, NCTC clone 929)
Lot Number:	70001022
Passage:	V2
Source:	American Type Culture Collection (ATCC), Manassas, VA

Table 1: Supplies

Supplies	Lot Number	Manufacturer	Expiration Date
Single Strength Minimum Essential Medium with 5% serum (MEM)	1X MEM 202	Pacific BioLabs	11/13/17
Dulbecco's Phosphate Buffered Saline (PBS)	63967517	ATCC	11/2018
Trypsin-EDTA	1837659	Life Technologies	10/2018

3.3. Experimental Design

3.3.1. Preparation of Media

Minimum Essential Medium (MEM) was prepared in accordance with Pacific BioLabs SOPs. The MEM was a 5% serum supplemented cell culture medium comprised of 93% Single Strength Minimum Essential Medium with Earle's salts (1XMEM), 5% Horse Serum, 1% Penicillin-Streptomycin (10,000 units/mL), and 1% solubilized Amphotericin B (250 µg/mL).

3.3.2. Preparation of Cell Cultures

Multiple cultures of L-929 mouse fibroblast cells (ATCC cell line CCL 1, NCTC clone 929) were prepared in accordance with Pacific BioLabs SOPs. Once a near confluent monolayer (greater than 80%) formed in the cell culturing flask, the cells were passed. This was done by first decanting the growth medium and rinsing the cells with PBS. The PBS was decanted and 0.25% (w/v) Trypsin-0.53 mM EDTA was added. The Trypsin-EDTA was allowed to remain in contact with the cells for 3 – 10 minutes until approximately 50% of the cells were detached and rounded. The flask was then rinsed with fresh growth medium and then cells were transferred to a sterile centrifuge tube. The tube was centrifuged at approximately 1250 rpm for 5 – 10 minutes. The medium was decanted off and the cells were re-suspended and counted using a hemocytometer. Once the cell density was determined, the cells were diluted to seeding density of 1.6×10^5 cells/mL and plated. The cell cultures were incubated at 37 ± 1°C with 5 ± 1% CO_2 for 24 – 48 hours prior to applying the test article in order for a uniform, near confluent cell monolayer to form in the wells.

3.3.3. Test and Control Article Description, Preparation, and Extraction

Test Article Description: The test articles, "MRIaudio Ear-Tips," were soft blue cones.

Test Article Preparation: The test article preparation and extraction conditions are presented in Tables 2 and 4. Six pieces were used in the extraction. The total surface area for six pieces was 66.60 cm^2 and was extracted at a ratio of 60 cm^2/20 mL (wall thickness was greater than 0.05 cm), yielding a volume of 22.2 mL. The test articles were made of an absorbent material, so the absorbing capacity of six pieces was measured and determined to be 11.4 mL. The absorbed volume was added to the calculated volume, resulting in a total extraction volume of 33.6 mL.

Test Article Extraction: Minimum Essential Medium (MEM) was used as the extractant. The extractions were performed according to Pacific BioLabs SOPs. The test articles were cut into smaller pieces and placed in a sterile glass container. Extraction medium was added and the test articles initially floated. Sterile borosilicate glass beads were added to weigh down the test articles and ensure they were completely immersed. Before extraction, the solution appeared normal (dark pink, clear, and free of particulates). The test article was incubated for 24 ± 2 hours at 37 ± 1°C with agitation.

Control Article Descriptions, Preparation, and Extraction: The control article preparation and extraction conditions are presented in Tables 3 and 4. USP High-Density Polyethylene Reference Standard is a white plastic and was used as the negative control. Tygon F-4040-A is a yellow plastic tubing and was used as the positive control. The control articles were cut into smaller pieces, placed into separate sterile glass containers, and immersed in appropriate volumes of extraction medium. A sufficient amount of MEM without test or control articles was placed in a sterile glass container and served as the reagent control. All control solutions appeared normal (dark pink, clear, and free of particulates). The control articles were incubated for 24 ± 2 hours at 37 ± 1°C with agitation.

Table 2: Test Article Extraction Volumes and Conditions

Total Surface Area (cm^2)	Extraction Ratio (cm^2/mL)	Calculated Volume (mL)	Absorbing Capacity (mL)	Total Volume Extracted (mL)	Extraction		
					Extraction Medium	Temperature (˚C)	Duration (hrs)
66.60	60/20	22.2	11.4	33.6	MEM	37 ± 1	24 ± 2

Table 3: Control Article Extraction Volumes and Conditions

Article	Total Surface Area (cm^2)	Extraction Ratio (cm^2/mL)	Total Volume (mL)	Extraction		
				Extraction Medium	Temperature (˚C)	Duration (hrs)
Positive Control	37.4	60/20	12.5	MEM	37 ± 1	24 ± 2
Negative Control	34.0	60/20	11.3			
Reagent Control	N/A	N/A	20.0			

N/A = Not Applicable

Table 4: Test and Control Article Extraction Dates and Times

Extraction Date (In)	Time (In)	Extraction Date (Out)	Time (Out)
November 07, 2017	1735	November 08, 2017	1647

3.4. Test Procedure

Plating of Test and Control Extracts: Following the 24 ± 2 hours extraction period, each extractant was allowed to come to room temperature, thoroughly mixed, and visually inspected. There was a color change noted in the test article extract; the solution turned pale pink. There were no observed changes in the color or clarity of the control article extracts and no particulate matter was present in any of the solutions. The test article and control extracts were stored at room temperature and administered to the cells within 24 hours after completion of the extraction process. The extracts were used undiluted, unfiltered, and were not manipulated in any way prior to dosing.

The growth medium from triplicate 10 cm^2 wells, each containing a monolayer of L-929 mouse fibroblast cells (ATCC Cell Line CCL1, NCTC Clone 929), was decanted and replaced with 2 mL of 1X Dulbecco's Phosphate Buffered Saline (PBS) to rinse away any debris. The PBS was then decanted and replaced with 2 mL of the test article extract in each well. The control extracts were administered in the same manner as the test article extract. All cell culture wells were then incubated for 48 ± 2 hours at 37 ± 1°C in a humidified incubator with 5 ± 1% CO_2. Since there was a color change noted in the test article extract, the pH was measured with pH indicator papers after dosing and determined to be approximately 8.0.

Cell Examination: The cells in all of the culture wells were examined under an inverted light microscope with 100X magnification after a minimum of 24 hours and 48 ± 2 hours following the dose administration.

3.5. Scoring Criteria

The conditions of cell cultures were graded according to criteria in Table 5. Only the average score for the three test wells at the 48-hour point was used to determine the final cytotoxic response of the test article. The average score for the controls at the 48-hour point was used to compare results.

Table 5: Qualitative Morphological Grading of Cytotoxicity of Extracts

Grade	Reactivity	Conditions of all Cultures
0	None	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.
1	Slight	Not more than 20% of the cells are round, loosely attached and without intracytoplasmatic granules, or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable.
2	Mild	Not more than 50% of the cells are round, devoid of intracytoplasmatic granules, no extensive cell lysis; not more than 50% growth inhibition observable.
3	Moderate	Not more than 70% of the cell layers contain rounded cells or are lysed; cell layers not completely destroyed, but more than 50% growth inhibition observable.
4	Severe	Nearly complete or complete destruction of the cell layers.

Table adopted from ISO 10993-5: *Biological Evaluation of Medical Devices – Part 5: Tests for In Vitro Cytotoxicity*

4. INTERPRETATION AND ANALYSIS

The test interpretation for the qualitative morphology evaluation is based on the cytotoxicity grade/reactivity grading scale listed in Table 5. The achievement of a numerical grade greater than 2 (mild reactivity) is considered a cytotoxic effect.

If the cultures treated with the test article show a significantly greater reaction than the cultures treated with the negative control, the client may request quantitative dilutions.

Validity: The test system is considered valid if the observed effect on the cells treated with the extracts prepared from the positive and negative controls react as follows: cells exposed to the positive control extract should score at least moderate reactivity (grade 3) and cells exposed to the negative control should score no reactivity (grade 0). If the controls do not have the expected response in the test system, the test will be repeated.

4.1. Statistical Analysis

No statistical analyses were performed.

4.2. Data Acquisition and Analysis

Major computer software systems used on this study included Microsoft Word® and the Rees Scientific Environmental Monitoring System® and/or the Vaisala Environmental Monitoring System® for environmental control of chambers used in this study.

4.3. Maintenance of Raw Data, Records, and Specimens

Following issuance of the Final Report, records (including, but not limited to, protocol, protocol amendment(s), in-life records, pathology records, dose preparation records, correspondence related to the study, Final Report, and histopathology records) and materials (including, but not limited to, slides, specimens, wet tissues, and blocks) will be archived at Pacific BioLabs (Hercules, CA) for a period of one year after issuance of the Final Report. After one year, the Sponsor will be contacted concerning continued storage or return of materials.

Records and materials associated with activities external to Pacific BioLabs (including, but not limited to, clinical pathology, and histopathology) and activities conducted by the Sponsor will be archived by the individual performing laboratories or the Sponsor in a manner consistent with their individual operating SOPs and regulatory requirements.

5. RESULTS AND DISCUSSION

5.1. Results

Scoring: Test results for the test article, positive, negative, and reagent controls are presented in Table 6. Cells treated with the test article extract exhibited a response of grade 1 (slight reactivity) at 24 hours and a response of grade 2 (mild reactivity) at 48 hours. Cells treated with the negative control extract and reagent control each exhibited a response of grade 0 (no reactivity) at 24 and 48 hours. Cells treated with positive control extract exhibited a response of grade 3 (moderate reactivity) at 24 hours and a response of grade 4 (severe reactivity) at 48 hours.

Validity: The test was considered valid because the test system reacted to the positive and negative controls as expected.

Table 6: Test Results

Replicate Number	Reactivity 24 hrs	Grade 24 hrs	Reactivity 48 hrs	Grade 48 hrs
Test Article #1	Slight	1	Mild	2
Test Article #2	Slight	1	Mild	2
Test Article #3	Slight	1	Mild	2
Controls				
Positive #1	Moderate	3	Severe	4
Positive #2	Moderate	3	Severe	4
Positive #3	Moderate	3	Severe	4
Negative #1	None	0	None	0
Negative #2	None	0	None	0
Negative #3	None	0	None	0
Reagent #1	None	0	None	0
Reagent #2	None	0	None	0
Reagent #3	None	0	None	0

ISO Scoring Key: 0=None (No Reactivity); 1=Slight Reactivity; 2=Mild Reactivity; 3=Moderate Reactivity; 4=Severe Reactivity
ISO Standard Interpretation: The achievement of a numerical grade greater than 2 is considered a cytotoxic effect

6. CONCLUSION

This study was conducted according to ISO 10993-5:2009. Based on qualitative evaluation of the cells exposed to the test article extract, the test article was not considered to have a cytotoxic effect (mild reactivity).

7. REFERENCES

ANSI/AAMI/ISO 10993-5:2009, *Biological Evaluation of Medical Devices – Part 5: Tests for In Vitro Cytotoxicity*

ISO 10993-12:2012, *Biological Evaluation of Medical Devices – Part 12: Sample Preparation and Reference Materials*

Pacific BioLabs SOP 15B–01, rev. 8G.01, *Cytotoxicity – Elution Test (ISO Method)*

APPENDIX I

Certificates of Analysis for Control Articles

Certificate

HIGH-DENSITY POLYETHYLENE

USP Catalog No.:	1546707
USP Lot No.:	K0M357

CAS No.:	9002-88-4
Molecular Formula:	CH_3-(CH_2-CH_2)$_n$-CH_3
Molecular Weight:	N/A

USP Certificate
Page 1 of 2

Certificate Date: 07Apr2015
USP Template No. CERT1_4-03
Effective 26Jan2015

U.S. Pharmacopeial Convention

LABEL TEXT

For use with specified USP compendial tests. Not for use as a drug. See SDS prior to use at www.usp.org/sds.

USP REFERENCE STANDARD

HIGH-DENSITY POLYETHYLENE

(3 strips, 2" x 2½" each)

Exercise care in handling and storage to avoid scratching the smooth surfaces of the strips.

USP, 12601 Twinbrook Pkwy, Rockville, MD, +1-301-881-0666
CAT. NO. 1546707 Material mfd. in United States

LOT: K0M357

Teri L. Toth

Quality Assurance

Calculation Value
If a value is not provided on the label or accompanying documentation and the Reference Standard has a quantitative USP compendial application, a value of 100.0% is used. The purity value is not applicable for qualitative uses. Please refer to the specific Reference Standard label for further information.

Expiration
Current lots are identified in the current USP Catalog. In some cases, the previous lot may still be considered valid for use. If so, it is identified in the column marked "Previous Lot/Valid Use Date."

It is the responsibility of each user to determine that this lot is current or valid when used. For the most up-to-date information, please refer to the USP Store at www.usp.org.

Instructions for Use
Follow the instructions on the label of the USP Reference Standard and in the appropriate USP documentary standard(s).

Non-Monograph Use
The suitability of this Reference Standard for use in non-compendial applications is solely the responsibility of the user.

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USP Certificate
Page 2 of 2

Certificate Date: 07Apr2015
USP Template No. CERT1_4-03
Effective 26Jan2015

SAINT-GOBAIN

CERTIFICATION

To: VWR INTERNATIONAL
BATAVIA, IL

SAINT-GOBAIN ORDER NUMBER: 96039741
CUSTOMER ORDER NUMBER: 4506648936

Line #	Customer Part Number	Formulation	Size	Quantity / U.M.
1	AAG00036 CUST #: 63010-450	TYGON® F-4040-A	½"ID x 5/8"OD x 50FT	1 / 50FT
Lot Numbers: 21926463		Mfg. Date: 11/22/2016		Shelf Life: 1 Year
Ship Date: 2/2/2017				

We hereby certify that the material listed above complies in full with the following requirements:

THE ABOVE COMPLIES IN FULL WITH OUR MANUFACTURING SPECIFICATIONS FOR THIS FORMULATION AND SIZE EXTRUSION(S).

Date: Wednesday, February 22, 2017

By: JANE M. FRANCIS

Jane M. Francis

QUALITY SYSTEM SPECIALIST

Customer Service Rep: Frank Chanowski

Saint-Gobain Performance Plastics
2664 Gilchrist Rd. • Akron, OH 44305 • Tel: (330) 798-9240
www.plastics.saint-gobain.com

APPENDIX II

Protocol

PBL Pacific BioLabs
The Service Leader in Bioscience Testing

STUDY SPONSOR

MRIaudio
2720 Loker Ave W
Suite N
Carlsbad, CA 92010
United States

GLP Study Protocol

Cytotoxicity-MEM Elution Test (ISO 10993-5:2009)

Study Number

17J0417H-M01G

PERFORMING LABORATORY

Pacific BioLabs
551 Linus Pauling Drive
Hercules, CA 94547
United States

1. GENERAL INFORMATION

This GLP Protocol (Protocol) describes testing for test and control articles (TACA) submitted by the Sponsor in compliance with the Food and Drug Administration's Good Laboratory Practice (GLP) Regulations (21CFR Part 58). Pacific BioLabs will require a *Laboratory Service Request* (LSR) form with each TACA that details the characteristics of the TACA submitted for testing.

1.1. Study Number

17J0417H-M01G

1.2. Study Title

Cytotoxicity – MEM Elution Test (ISO 10993-5:2009)

1.3. Test Facility

Pacific BioLabs
551 Linus Pauling Drive
Hercules, CA 94547
Unites States

1.4. Responsible Personnel

Sponsor's Representative:

Joseph Caruso
MRIaudio
2720 Loker Ave W
Suite N
Carlsbad, CA 92010
United States

Phone: 858-266-8350
e-mail: joe@mriaudio.com

Study Director:

Jessica Renfandt
Phone: (510) 964-9000
e-mail: jessicarenfandt@pacificbiolabs.com

1.5. Proposed Study Dates

The study dates may change due to unexpected events and major delays in the study conduct will be communicated with the Sponsor. The actual study dates will be specified in the Study Report and will not be added by amendment to the Protocol.

Proposed Start Date:	To Be Determined
Proposed Termination Date:	To Be Determined
Proposed Report Date:	To Be Determined

1.6. Alterations to the Protocol

Alterations to the general scope of the Protocol may be made over the period that the Protocol is in effect. Alterations to the Protocol that apply to all subsequent testing will be documented by an amendment to the Protocol and signed and dated by Pacific BioLabs and the Sponsor. In the event that a protocol change is verbally authorized by the Sponsor, Pacific BioLabs will honor the change. However, written authorization from the Sponsor will be obtained thereafter. All Protocol amendments will be issued to the Sponsor and will be included in the Study Report. Administrative protocol changes may not require Sponsor signature.

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All deviations to the Protocol during the course of the study will be justified by the Study Director as to impact on the study. All deviations and justification of the impact will be documented in the Study Report.

1.7. Statement of Compliance

This nonclinical laboratory study will be conducted in accordance with the appropriate Standard Operating Procedures of Pacific BioLabs (Hercules, CA) and the Food and Drug Administration Good Laboratory Practice (GLP) Regulations For Nonclinical Laboratory Studies (21 CFR Part 58). This nonclinical study will be inspected by the Quality Assurance Unit (QAU) at Pacific BioLabs at intervals adequate to assure the integrity of the study. QAU inspection findings will be reviewed by the management of Pacific BioLabs; and the Study Director and management will be notified immediately if there are any deviations which might affect the integrity of the study data.

Supporting Studies Conducted by Pacific BioLabs Designated Laboratories: There are no supporting studies conducted by outside laboratories, designated by Pacific BioLabs that contribute to this Protocol.

Supporting Studies Conducted by Sponsor: This Protocol does not incorporate supporting studies conducted by the Sponsor. All studies conducted by the Sponsor in conjunction with this Protocol will be reported separately by the Sponsor and will be the sole responsibility of the Sponsor.

1.8. Safety to the Laboratory

The Sponsor will provide safety information to Pacific BioLabs in the form of a Safety Data Sheet (SDS) for each test article, if available. In the absence of specific safety requirements, standard laboratory safety procedures will be employed for handling the test and control articles, including the use of appropriate personal protective equipment.

1.9. Declaration of Intent

The design and scope of this study are consistent with the overall development strategy of the Sponsor, and this study may be submitted to regulatory agencies, including the United States Food and Drug Administration (FDA).

2. PURPOSE

The purpose of this study is to evaluate the cytotoxic potential of extracts of polymeric materials or any other materials intended to be implanted in the human body or that may come into contact with the intact skin, bodily fluids or injectable solutions. This test will be performed according to Pacific BioLabs SOP 15B-01, which follows procedures outlined in ISO 10993-5:2009.

2.1 Justification of Test System

Mouse fibroblast L-929 cells (ATCC cell line CCL 1, NCTC clone 929) were selected based on recommendation by ISO 10993-5 guidelines.

2.2 Justification of Route of Administration

The route of administration was based on ISO 10993-5 requirements for this test. The test article will be extracted and administered to mouse L-929 cells via medium compatible with the test system.

2.3 Justification of Selection of Media

Single Strength Minimum Essential media was selected based on recommendations provided by ISO 10993-5.

2.4 Dose Rationale

The dose was selected based on recommendations provided by ISO 10993-5 guidelines.

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3. PROCEDURES

3.1. Test Materials

3.1.1. Test and Control Articles

Identification and characterization of test articles will be specified in the Study Report of test results. The following information, supplied by the Sponsor, may be included in the Study Report:

Test Article Name:	MRIaudio Ear-Tips
Physical Description:	Solid
Lot Number:	300-1
Sample Code:	Not provided by Sponsor
Part Number:	350
Expiration Date:	10/01/2018
Special Handling and/or Precautions:	None
Sterilization Data:	Non-sterile
Storage Conditions:	Room Temperature
Final Intended Use:	Used as an earbud for sound protection

3.1.2. Test Article Identification

Control Articles will be provided by Pacific BioLabs and will be specified in the Final Report as follows:

3.1.3. Negative Control Article Identification

Negative Control Article Name:	USP High-Density Polyethylene (HDPE) Reference Standard
Physical Description:	Plastic
Manufacturer:	USP
Lot Number:	Will be provided in the Final Report
Sterility Status:	Will be autoclaved at Pacific BioLabs
Expiration Date:	Will be provided in the Final Report
Special Handling and/or Precautions:	None
Storage Conditions:	Room Temperature

3.1.4. Positive Control Article Identification

Positive Control Article Name:	Tygon F-4040-A
Physical Description:	Solid; Yellow Plastic Tubing
Manufacturer:	Saint-Gobain Performance Plastics
Lot Number:	Will be provided in the Final Report
Sterility Status:	Non-Sterile
Expiration Date:	Will be provided in the Final Report
Special Handling and/or Precautions:	None
Storage Conditions:	Room Temperature

Test and Control Article Characterization: The Sponsor will supply Certificates of Analyses and stability certifications for GLP required characterization of the purity, composition, stability and other pertinent information for the test and control article(s). Similar information for materials (e.g., excipients) used in preparation of dose solutions, if applicable, will be obtained by Pacific BioLabs. Documentation of the characterization of test articles, control articles and excipients (as applicable) will be included in the Study Reports. The absence of documentation of the identity, composition, strength and stability of the test articles or control articles (e.g., a CofA) will be considered noncompliance with GLP expectations and will be documented in the Final Report.

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The Sponsor's signature and approval of this Protocol indicates that appropriate documentation of the method of synthesis, fabrication or derivation of the test and control article(s) is available to the appropriate regulatory agencies if requested.

Dose Formulation Analysis: Dose formulation analysis will not be conducted for prepared test articles.

Reserve Sample and Sample Disposition: Unless requested otherwise, unused test articles or control articles will be discarded or destroyed at the end of the study according to Pacific BioLabs SOPs.

FDA and US Environmental Protection Agency (EPA) regulations require that, for studies of more than four weeks duration, reserve sample from each batch of material be retained for the period of time provided in FDA GLP Regulations 21 CFR Parts 58.105 and 58.195; EPA FIFRA GLP Regulations 40 CFR Parts 160.105 and 160.195; and EPA TSCA GLP Regulations 40 CFR Parts 792.105 and 792.195. The various agencies have, in the past, recommended that the amount of reserve sample be enough to repeat the study two or three times. Sponsor is responsible for retention of test and control article reserves.

3.2. Test System

Name:	Fibroblast cell
Organism:	*Mus musculus*
Cell Line:	L-929 (ATCC cell line CCL 1, NCTC clone 929)
Lot Number:	Will be provided in the Final Report
Passage:	Will be provided in the Final Report
Source:	American Type Culture Collection (ATCC), Manassas, VA

3.3. Experimental Design

Preparation of Cell Cultures: Multiple cultures of L-929 (ATCC cell line CCL1, NCTC clone 929) mouse fibroblast cells will be prepared in accordance with Pacific BioLabs SOPs, using a 5% serum supplemented cell culture medium (MEM). The cell cultures will be plated 24-48 hours prior to applying the extract medium in order to allow for a uniform, near confluent (greater than 80% confluent) cell monolayer to form in the wells.

Test Article Preparation: The test material will be prepared based on Sponsor requirements, and performed according to Pacific BioLabs SOPs, which follow methods found in ISO 10993-12. MEM will be used as the extractant and the extraction conditions will be 37 ± 1°C for 24 ± 2 hours, with agitation. Extraction volume will be calculated based on the surface area of the test article, as specified in ISO 10993-12 guidelines. Positive and negative controls will also be prepared. Extracts will be stored at room temperature and used within 24 hours after the completion of the extraction process.

Procedures: Triplicate cell culture wells will be used for the test samples and controls (positive, negative and reagent controls). A vial containing 20 mL of MEM will serve as the reagent control. After the 24 ± 2 hours extraction period, the growth medium from triplicate 10 cm^2 wells, each containing a monolayer of L–929 mouse fibroblast cells will be decanted and replaced with 2 mL of 1X Phosphate Buffered Saline (PBS) to rinse away debris. The PBS will be decanted and replaced with 2 mL of test article extract. The control solutions will be tested in the same manner as the test article extract. All cell culture wells will then be incubated for 48 ± 2 hours at 37 ± 1°C in a humidified incubator with 5 ± 1% CO_2.

The cells in all of the culture wells will be examined under an inverted light microscope with 100X magnification at two observation periods (after a minimum of 24 hours and at 48 ± 2 hours) following dose administration. The morphology of the cells will be scored at the 24 and 48 hour examination points according to the criteria stated in the ISO 10993-5 guidelines. The average score for the three cell culture wells treated with the test article extract at the 48 hour observation point will be used to determine the final cytotoxic response. The average score for the controls at the 48 hour point will be used to compare results.

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Interpretation: As recommended by ISO, the test interpretation for qualitative morphology will be based on a grade/reactivity scale (Table 1). The achievement of a numerical grade greater than 2 is considered a cytotoxic effect. The test system will be considered valid if the observed effect on the cells treated with the extracts prepared from the positive and negative controls react as follows: cells exposed to the positive control extract should score at least Moderate Reactivity (grade 3) and cells exposed to the negative control should score No Reactivity (grade 0). If the controls do not have the expected response in the test system, the test will be repeated.

Test interpretation for the qualitative evaluation will be based on the cytotoxicity scale provided by the ISO guidelines and Pacific BioLabs SOPs (Table 1). If the cultures treated with the test article show a significantly greater reaction compared to the cultures treated with the negative control, the client may request quantitative dilutions.

Table 1: Qualitative Morphological Grading of Cytotoxicity of Extracts

Grade	Reactivity	Conditions of All Cultures
0	None	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.
1	Slight	Not more than 20% of the cells are round, loosely attached and without intracytoplasmatic granules, or show change in morphology; occasional lysed cells are present; only slight growth inhibition observable.
2	Mild	Not more than 50% of the cells are round, devoid of intracytoplasmatic granules, no extensive cell lysis; not more than 50% growth inhibition.
3	Moderate	Not more than 70% of the cell layers contain rounded cells or are lysed; cell layers not completely destroyed, but more than 50% growth inhibition observable.
4	Severe	Nearly complete or complete destruction of the cell layers.

*Table adopted from ISO 10993-5:2009 Biological Evaluation of Medical Devices- Tests for *In Vitro* Cytotoxicity

4. DATA ACQUISITION AND ANALYSIS

4.1. Descriptive Statistics

No descriptive statistics will be generated by Pacific BioLabs for this study.

4.2. Statistical Analysis

No statistical analyses will be performed by Pacific BioLabs for this study.

5. REPORTS

5.1. General Description of Study Report

The Study Report will include all information necessary to provide a complete and accurate description of the experimental procedures and results. The Study Report will include a compliance statement signed by the Study Director that the report accurately reflects the raw data obtained during the performance of the study and that all applicable GLP regulations were followed in the conduct of the study.

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5.2. Study Report

The Study Report will include, but not be limited to, the following:

Name and address of the test facility
Study dates
Study summary
The objective of the study
Test and control article identification
A full description of the test system
A full description of the experimental design and methods
Study results in prose and tabular form as appropriate
Any deviations from the Protocol
Signed statement of compliance from the Study Director

The Study Report will not include results of analyses performed by the Sponsor. Communication of the results of these Sponsor-conducted analyses to the appropriate regulatory agencies will be the responsibility of the Sponsor. Upon finalization, copies of the Final Reports will be provided to the Sponsor as hardcopies or PDF files.

6. MAINTENANCE OF RAW DATA, RECORDS AND SPECIMENS

Original data, specimens and reports from this study are the property of the Sponsor. These materials will be available to the Sponsor to facilitate reviewing the study during its progress and before issuance of the Final Report. Records (including, but not limited to, protocol, protocol amendments(s), dose preparation records, correspondence related to the study, Final Report, and materials (including, but not limited to, slides, specimens, wet tissues and blocks) will be archived at Pacific BioLabs (Hercules, CA) for a period of one year after issuance of the Final Report. After one year, the Sponsor will be contacted concerning continued storage or return of materials.

Records and materials associated with activities external to Pacific BioLabs (including, but not limited to, clinical pathology, histopathology, and bio-analysis) and activities conducted by the Sponsor (including, but not limited to, dose solution analysis), will be archived by the individual performing laboratories or the Sponsor in a manner consistent with their individual operating SOPs and regulatory requirements.

7. REFERENCES

Good Laboratory Practice Regulations; Food and Drug Administration: 21 CFR Part 58.
Good Laboratory Practice Regulations; Environmental Protection Agency: 40 CFR Part 160
ANSI/AAMI/ISO 10993-5:2009, *Biological Evaluation of Medical Devices-Part 5: Tests for In Vitro Cytotoxicity*.
ISO 10993-12:2012, *Biological Evaluation of Medical Devices-Part 12: Sample Preparation and Reference Materials.*
PBL SOP 15B-01, rev. 8G.01, *Cytotoxicity – Elution Test (ISO Method)*

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8. APPROVALS

FOR SPONSOR

Study Sponsor — Date: 10/30/17

FOR PACIFIC BIOLABS

Jessica Renfandt
Study Director — Date: NOV. 06 2017

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